ACTIVITIES OF MESENCHYME IN CERTAIN LARVAE.

CHARLES B. WILSON.

In consideration of the attention which has recently been given to a study of the phenomena of the living cell, the following observations may be found of some interest. They were made during the summers of 1896 and 1897 upon mesenchyme cells in the larvae of certain species of molluscs and nemerteans.

Freshly laid eggs of the nudibranch mollusc *Tergipes despectus* (?) were obtained and reared to the veliger stage. Both egg and young embryo are nearly opaque, the gastrula invagination being distinguished only by a slight increase in the density. But after the mantle has been formed and the shell has been secreted, the veliger becomes perfectly transparent. The delicate shell is as clear as the finest glass, and the whole internal structure is now plainly visible.

Mesenchyme cells appear very early in development. They arise from large primitive endoderm cells at the posterior edge of the blastopore in the ordinary way for gastropod molluscs, and are set free in the segmentation cavity. At first they are approximately spherical in shape, and float about freely in the liquid which fills the cavity, but they soon begin to elongate, and become spindle-shaped. At this stage in their development activities were observed which are probably analogous to those described for the polar bodies of certain animals.[1]

Unfortunately, I did not have with me at the time an objective of sufficient power to bring out the finer protoplasmic movements distinctly. Well-defined amoeboid changes of outline, however, could be plainly seen. The cell, as it moves about in the liquid which fills the segmentation cavity, puts out blunt pseudopodia-like processes. These change both their

[1] Andrews, E. A., "Some Activities of Polar Bodies," *Johns Hopkins University Circular.* November, 1897.

shape and position. There seems to be a special tendency to their formation whenever the cell comes close to the wall of the cavity, or when two cells approach each other. The processes remain short and blunt, and in no instance were they seen to reach either the wall or a neighboring cell. The putting out of these processes was seen to be accompanied in several instances by corresponding movements of the cell contents very similar to those in an amoeba.

Certain disturbances were also noticed in the liquid close to the surface of the cell. At the time these were considered analogous to slow ciliary motion, and they were probably caused by "filose" action.

It was impossible to determine with certainty whether the amoeboid changes ceased and were followed by a period of rest before the cell became permanently branched; but this would seem probable from analogy.

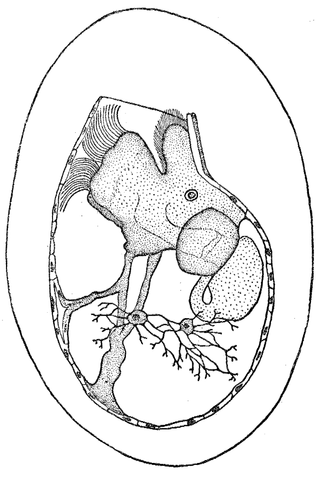

FIG. 1. — Side view of veliger larva of Tergipes, showing position of the two contractile cells. Cam. luc. drawing, Leitz objective No. 3, Eye-piece No. 3.

At all events, permanent processes soon appear, the cells become fastened in place, and after subsequent development function as muscles.

During the development, after the cells become branched, certain activities appear which are of an entirely different nature.

A single cell, or more often a pair of these mesenchyme cells, may be found in close proximity to the internal wall of the mantle on one (usually the right) side nearly in the center (Fig. 1).

These two cells are in close proximity to each other, and each consists of a body and radiating branches. The body is composed of finely granular protoplasm, and contains a distinct nucleus and several vacuoles. From it branches extend in many different directions. There are usually several fine branches reaching directly across from the body of one cell to

that of the other, which serve to bind the cells firmly together. The other branches are at first of about the same size, but one branch from each cell, extending outward (*i.e.*, away from the twin cell), becomes very quickly larger than the rest, sometimes approaching the diameter of the cell itself. These two larger branches are seldom in the same straight line, but usually make a more or less obtuse angle with each other. That they are really branches and not simply a prolongation of the cell body is apparent from the fact that they are finely fibrous in structure like all the other branches, and not granular like the body of the cell.

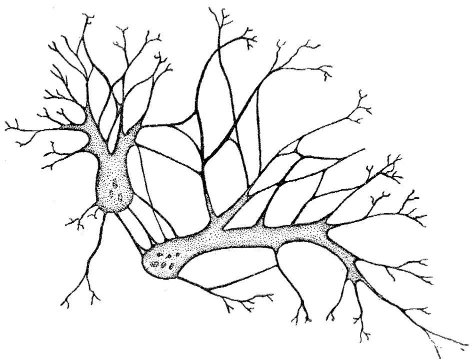

FIG. 2. — The two contractile cells enlarged to show their structure. Cells relaxed. Cam. luc. drawing, Leitz objective No. 7, Eye-piece No. 3.

These large branches extend some distance from the cell and then divide dichotomously, ending in fine fibrils.

The smaller divisions of these branches, together with those of the cell itself, anastomose freely and form a loose reticular network. These details of structure appear to better advantage in the enlarged drawing of the two cells shown in Fig. 2.

At this stage of their development they are not attached to anything except to each other, but the network formed by their interlacing branches extends over a considerable area and holds them in position.

They appear like ordinary mesenchyme cells, but upon being watched they are seen to possess a peculiar contractile power, which is manifested at intervals. In a few individuals the contractions occurred at definite intervals as long as the cell was watched, but more frequently there was a period of rest after a few contractions. Both the time of contracting and the intervals of rest were subject to considerable variation, but the latter was never long enough to enable a camera lucida sketch

to be made. The drawings in Figs. 1 and 2 were taken from individuals which had been paralyzed with magnesium sulphate.

These cells are isolated from everything except the liquid in which they lie, and, consequently, if there be any stimulus previous to contraction, it must be given through the medium of the liquid or it must arise spontaneously in the cell itself.

By careful watching, the contraction can be seen to begin in the cell body and travel outward along the branches, though the contractile wave moves so quickly that it practically begins at all points simultaneously. As a result of its action the protoplasm draws together, the cell body becomes more spherical, all the branches, large and small alike, become shorter and thicker, and the whole meshwork of fibrils is drawn in until it occupies much less area than formerly.

When it first begins, the contraction is comparatively weak and results simply in a shortening of the branches and fibrils, but as it proceeds it becomes rapidly stronger and stronger. This increase in contraction cannot manifest itself in any further shortening of the branches, for they have already shortened all they are capable of doing. The only way in which the two ends of any branch can now be brought nearer together is by a bending or folding of the fibers upon themselves, and this is what actually occurs. At the close of contraction (Fig. 3) the smaller branches and fibrils have been drawn in so much that they are twisted into a corkscrew shape for their entire length. The large branches have also contracted so strongly that their surface becomes wavy or sinuous in outline. The bodies of the cells remain spherical, but become so opaque that neither nucleus nor vacuoles are visible.

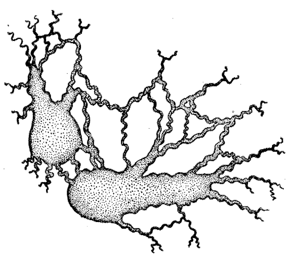

FIG. 3. — The same two cells at the close of contraction. Leitz objective No. 7, Eye-piece No. 3.

This twisting or plication of the muscle fibers, whereby their retractive power is increased, is also shown in the retractor muscle of the velum (Fig. 1), and will be noticed later in certain muscles of the nemertean larva. The same thing has

been observed in "single fibrils in protoplasm, as well as contractile pellicles and substance membranes," and also in Metazoan cilia and the muscle bands in rotifers.[1] It seems to be carried farther here than in the muscles mentioned, for the simple reason that these filaments are unattached and, therefore, there is nothing to restrain it.

After remaining an instant in this extreme contraction, the cells relax, and the return to a normal condition is practically instantaneous. We have here, then, the same power manifested by the single mesenchyme cell, with its branches, that belongs to the more complicated retractor muscle, and that, too, when it is isolated from everything save the liquid in which it floats. This must certainly be a very near approach to a primitive muscular contractility.

The contraction lasts one and a half or two seconds, the relaxation occupies but a very small fraction of a second, while the pause or rest varies from two or three to twelve or fifteen seconds. This suggests very forcibly a condition similar to that which obtains in the beating of the heart, with the exception that in these mesenchyme cells the relative duration of contraction and relaxation is reversed, the former being much longer.

This same contractile power is also possessed, to a less degree, by the other mesenchyme cells. They may often be seen to contract after they have become branched. The contractions are not as rhythmical as those just described, but they are as automatic. Some of these mesenchyme cells enter the velum during development and become attached to its walls until the whole interior is traversed from wall to wall by their branches, rendering it highly contractile.[2]

In this case, therefore, the same cell which contracts at first automatically may afterward become a part of the muscular network of the velum, where it is under the control of the central nervous system.

Similar phenomena were observed in the mesenchyme cells of the pilidium larvae of the Nemertean *Cerebratulus lacteus*

[1] Andrews, G. F., The Living Substance as Such: and as Organism, p. 103. 1897.

[2] Lang, Comparative Anatomy, Part II, p. 257.

Verrill. These larvae were reared from artificially fertilized eggs, and a full account of their development is in preparation for a subsequent paper. The eggs of this nemertean are opaque during cleavage and gastrulation, but become beautifully transparent on reaching the pilidium stage.

The mesenchyme first appears as isolated cells derived from the ectoderm, as observed by Metschnikoff (*Zeit. f. wiss. Zool.*, Bd. xxxix).

They move about freely in the gelatinous liquid which fills the space between ectoderm and entoderm. At first they are nearly spherical in outline, but they soon begin to develop processes and become branched, in which condition they are very readily distinguished from the other elements. So long as they remain free floating there is no indication of cell fibers, but simply a nucleus enclosed in cytoplasm. But as they begin to branch they grow larger, and granules appear in the cytoplasm, while the branches become gradually fibrous in structure. No amitotic division stages, however, were noticed in any of these cells, such as were found by Montgomery in the free-floating mesenchyme cells of the adult worm (*Zool. Jahrb.*, Bd. x). The branches hinder the freedom of motion of the cells, and the latter gradually become fixed in position.

The fibrils at the extremities of the branches are then fastened in place, and from being mere wandering mesenchyme the cell becomes one of the muscles of the pilidium. This transformation was watched several times in the formation of different muscles, and nearly all the intermediate stages were observed. The most important muscle of the pilidium is the one which extends from the apical plate downward to the anterior border of the lappets. The development of this muscle was watched in many different individuals. When the mesenchyme cells first develop branches, one of them can be seen to become stationary in about the position of the future apical muscle. One of its processes becomes fastened to the apical plate, while another fastens to the wall of the digestive tract, and sometimes a third connects with the aboral wall of the pilidium (Fig. 4). The number of processes is not constant, but the position assumed by the cell is approximately so.

Other cells become fastened to the walls of the digestive tract along its anterior border. The branches of these cells anastomose with each other and with the first cell, and from them are developed the strong muscle which enables the larva to retract the apical plate with its tuft of cilia. This muscle becomes attached at first to the wall of the digestive tract, as figured by Verrill (*Marine Nemerteans of New England*, p. 417). But as soon as the branches begin to anastomose it

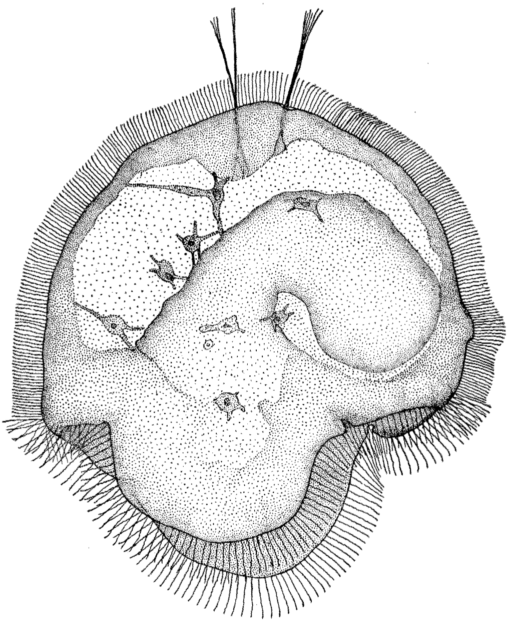

FIG. 4. — Side view of pilidium larva, showing mesenchyme cells in position to form the apical muscle. Zeiss cam. luc. × 575 diams.

develops along the line of mesenchyme cells seen in Fig. 4, and becomes fastened to the anterior border of the lappets. In a similar way a transverse muscle is formed just in front of the apical plate.

This consists of a single large mesenchyme cell which subsequently develops long processes reaching from one side of the pilidium to the other. In later development the whole internal surface of the umbrella is covered with a loose meshwork of anastomosed mesenchyme cells, which give the larva so much

contractile power that it frequently tears the umbrella cells apart by violent contractions when irritated.

While floating about freely these mesenchyme cells do not contract, so far as could be observed, but as soon as they begin to form processes they can be seen to contract. Occasionally two cells anastomose with each other before becoming attached to the wall of the pilidium.

In such a case they contract irregularly at first, the intervals between contractions being unequal, but later the contractions become rhythmical and very closely resemble those of the opisthobranch gastropods just described. Three or four contractions occur in rapid succession and are followed by a comparatively long rest. After the cell branches become fastened to the pilidium wall these rhythmical pulsations cease. The mesenchyme cells now become regular muscles of the larva and contract only when stimulated from the central nervous system.

We are witnessing here, then, the passage from an automatic condition, in which the cells contract quite independently from the rest of the larva, into a condition in which every contraction is definitely correlated with that of the other larval muscles.

In this nemertean larva the intense contractions resulting in a corkscrew shortening of the branches and fibers occurred subsequent to the fixation of the cells. It was not noticed in any of the free cells even when two of them anastomosed before becoming attached, and all the conditions appeared as favorable as in the veliger larvae. After attachment such a shortening is very noticeable, especially in the apical muscle and the fine radial muscles of the side lappets.

In an examination of these larvae, therefore, it is found that:

1. The mesenchyme cells are at first nearly spherical and are free-floating. In this condition they consist simply of a nucleus and cytoplasm; they may put out amoeboid processes, but they do not show any contractile movements.

2. They soon grow larger, become granular, and develop fibrous branches which hinder their free motion, and finally they become fixed in position and function as muscles.

3. In both larvae prior to such fixation cells may be found, singly or in pairs, which pulsate in more or less rhythmical contractions until their branches become fastened to the larval tissue, when the pulsations cease.

4. Both larvae, accordingly, show a well-marked transition from automatic pulsations to muscular contractions dependent upon the central nervous system.

State Normal School, Westfield, Mass.,
April 18, 1898.